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Determination of Hexachlorophene and  
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DETERMINATION OF HEXACHLOROPHENE AND DICHLOROPHEN IN  
INDUSTRIAL AND MUNICIPAL WASTEWATERS

by

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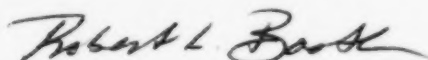
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## FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati, conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.
- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report is one of a series that investigates the analytical behavior of selected pesticides and suggests a suitable test procedure for their measurement in wastewater. The method was modeled after existing EPA methods being specific yet as simplified as possible.



Robert L. Booth, Director  
Environmental Monitoring and Support  
Laboratory - Cincinnati

## ABSTRACT

A method was developed for the determination of hexachlorophene and dichlorophen in wastewaters. The method development program consisted of a literature review; determination of extraction efficiency for each compound from water into methylene chloride; and determination of suitable liquid chromatographic analysis conditions.

The final method was applied to Columbus Publicly Owned Treatment Works (POTW) secondary effluent in order to determine the precision and accuracy of the method. The wastewater was spiked with the two compounds at levels of 10  $\mu\text{g/L}$  and 50  $\mu\text{g/L}$ . Recoveries for hexachlorophene at the 10  $\mu\text{g/L}$  and 50  $\mu\text{g/L}$  levels were  $82 \pm 3$  percent and  $102 \pm 6$  percent, respectively. Recoveries for dichlorophen at the 10  $\mu\text{g/L}$  and 50  $\mu\text{g/L}$  levels were  $58 \pm 12$  percent and  $107 \pm 4$  percent, respectively. The method detection (MDL) in reagent water for hexachlorophene was 1.2  $\mu\text{g/L}$  and for dichlorophen was 1.0  $\mu\text{g/L}$ . The MDL in wastewater may be higher due to interfering compounds.

This report was submitted in partial fulfillment of Contract No. 68-03-2956 by Battelle Columbus Laboratories under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from February 1, 1982 to April 30, 1982.

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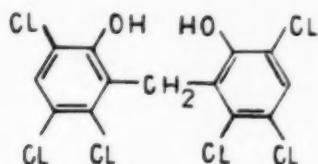
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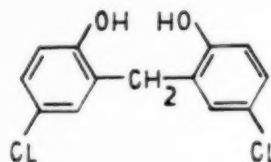
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SECTION I  
INTRODUCTION

Hexachlorophene (I) and dichlorophen (II) are used as bactericides and fungicides.



I



II

The CAS registry number for hexachlorophene is 70-30-4 and its CAS name is 2,2'-methylenebis[3,4,6-trichlorophenol]. It has a melting point of 164-165°C. Common synonyms for hexachlorophene include "Bilevon", "Dermadex", and "Exofene". The CAS registry number of dichlorophen is 97-23-4 and its CAS name is 2,2'-methylenebis[4-chlorophenol]. It has a melting point of 177-178°C and an oral LD50 in rats of 2.69 g/kg. Common synonyms for dichlorophen include "Kindar" and "Preventol GD".

A method for hexachlorophene has been reported in which the hexachlorophene was derivatized with N,O-bis-(trimethylsilyl)-acetamide (BSA) and analyzed by gas chromatography (GC) with flame ionization detection (FID) (1). Similar methods for dichlorophen have been reported using BSA (1) and N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) (2) as derivatizing reagents.

Hexachlorophene and dichlorophen are reasonably stable in water and can be extracted from water with methylene chloride. The selected approach to the determination of hexachlorophene and dichlorophen in water included separatory funnel extraction from water with methylene chloride, and analysis using high performance liquid chromatography (HPLC) with ultraviolet detection (UVD). Standard concentration techniques using Kuderna-Danish (K-D) equipment were used. The final method is included in Appendix A of this report.

## SECTION 2

### CONCLUSIONS

A method to determine hexachlorophene and dichlorophen in wastewaters was developed using an HPLC method of analysis with UVD.

### EXTRACTION AND CONCENTRATION

Hexachlorophene and dichlorophen can be extracted from reagent water into methylene chloride with greater than 90 percent recovery using separatory funnel techniques. Use of K-D concentration equipment to perform extract concentrations did not significantly decrease compound recoveries.

### CHROMATOGRAPHY

Two HPLC columns, Spherisorb-ODS and Lichrosorb RP-2, were found to be acceptable for this application. The Spherisorb-ODS column was used as the primary column. The Lichrosorb RP-2 column was designated as the alternate column.

### VALIDATION STUDIES

Recoveries of hexachlorophene and dichlorophen from reagent water in the 4 to 1000 µg/L concentration range averaged greater than 85 percent. The analytical curves constructed from these data were linear. The MDL in reagent water was 1.2 µg/L for hexachlorophene and 1.0 µg/L for dichlorophen. Recoveries of hexachlorophene from Columbus POTW secondary effluent were  $82 \pm 3$  and  $102 \pm 6$  percent at the 10 µg/L and 50 µg/L levels. Recoveries of dichlorophen were  $58 \pm 12$  and  $107 \pm 4$  percent at the same levels.

is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified<sup>3-5</sup> for the information of the analyst.

#### 5. Apparatus and Materials

##### 5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with Teflon. Aluminum foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

### SECTION 3

#### EXPERIMENTAL

Studies were performed to determine if extractions using separatory funnels, concentration using K-D equipment, and analysis using HPLC with UVD would be applicable techniques for the determination of hexachlorophene and dichlorophen in water.

#### EXTRACTION AND CONCENTRATION

Extraction of hexachlorophene and dichlorophen from water was studied using separatory funnel techniques. One liter of reagent water was used. The reagent water was adjusted to pH 12 by addition of 1 N sodium hydroxide. The reagent water was spiked with hexachlorophene and dichlorophen at the 10  $\mu\text{g/L}$  and 100  $\mu\text{g/L}$  levels and extracted three times with 60 mL each of methylene chloride. These extracts were discarded and 50 g of  $\text{NaH}_2\text{PO}_4$  were added to the water to adjust the pH to 4.0 to 4.5. The water was reextracted three times with 200 mL each of methylene chloride. The extracts were dried by passing them through 10 cm of anhydrous granular sodium sulfate, concentrated to a final volume of 5 mL and analyzed by HPLC. These studies were done in duplicate.

#### CHROMATOGRAPHY

Two HPLC columns were evaluated for the determination of hexachlorophene and dichlorophen: Spherisorb-ODS and Lichrosorb RP-2.

#### VALIDATION STUDIES

The MDLs for hexachlorophene and dichlorophen were determined by analyzing seven replicate reagent water samples spiked at the 4  $\mu\text{g/L}$  concentration level. The amounts recovered were determined by external standard calibration and the MDLs were calculated from these data.

Reagent water was also spiked in duplicate at the 4, 10, 50, 200, and 1000  $\mu\text{g/L}$  concentration levels and recoveries of the hexachlorophene and dichlorophen was determined as described earlier. An analytical curve was generated by plotting the amount spiked into the samples versus the amount recovered from the samples.

Columbus POTW water was used for wastewater validation studies. Seven replicates of the wastewater were analyzed to determine the background levels. The wastewater was spiked with hexachlorophene and

dichlorophen at the 10 and 50  $\mu\text{g/L}$  concentration levels, processed and analyzed. Seven replicate extractions were performed at each concentration level.

## SECTION 4

### RESULTS AND DISCUSSION

#### EXTRACTION AND CONCENTRATION

Hexachlorophene and dichlorophen were extracted from reagent water with greater than 85 percent recovery using separatory funnel techniques. Recoveries of hexachlorophene from water were 95 and 101 percent at 10  $\mu\text{g/L}$  and 91 and 99 percent at 100  $\mu\text{g/L}$ . Recoveries of dichlorophen were 81 and 97 percent at 20  $\mu\text{g/L}$  and 84 and 86 percent at 100  $\mu\text{g/L}$ . These data are the results of duplicate analyses.

#### CHROMATOGRAPHY

Both the Spherisorb-ODS and Lichrosorb RP-2 columns were satisfactory for the determination of hexachlorophene and dichlorophen. The Spherisorb-ODS column was chosen as the primary column (column 1) since it provided slightly better peak shape for the phenolic pesticides. The following conditions were used:

|                   |                                                                                  |
|-------------------|----------------------------------------------------------------------------------|
| Column:           | Spherisorb-ODS, 5 micron,<br>250 x 4.6 mm                                        |
| Solvent:          | 65 percent acetonitrile/35 percent<br>water/0.05 percent $\text{H}_3\text{PO}_4$ |
| Flow:             | 1 mL/min                                                                         |
| Detector:         | UV @254 nm                                                                       |
| Injection Volume: | 50 $\mu\text{L}$ .                                                               |

A chromatogram obtained under these conditions is shown in Figure 1.

The Lichrosorb RP-2 column was chosen as the alternate column (column 2). The following conditions were used:

|                   |                                             |
|-------------------|---------------------------------------------|
| Column:           | Lichrosorb RP-2, 5 micron, 250 x 4.6 mm     |
| Solvent:          | 60 percent acetonitrile/40 percent<br>water |
| Flow:             | 1 mL/min                                    |
| Detector:         | UV @254 nm                                  |
| Injection Volume: | 50 $\mu\text{L}$                            |

Chromatograms obtained under these conditions are shown in Figures 2 and 3.

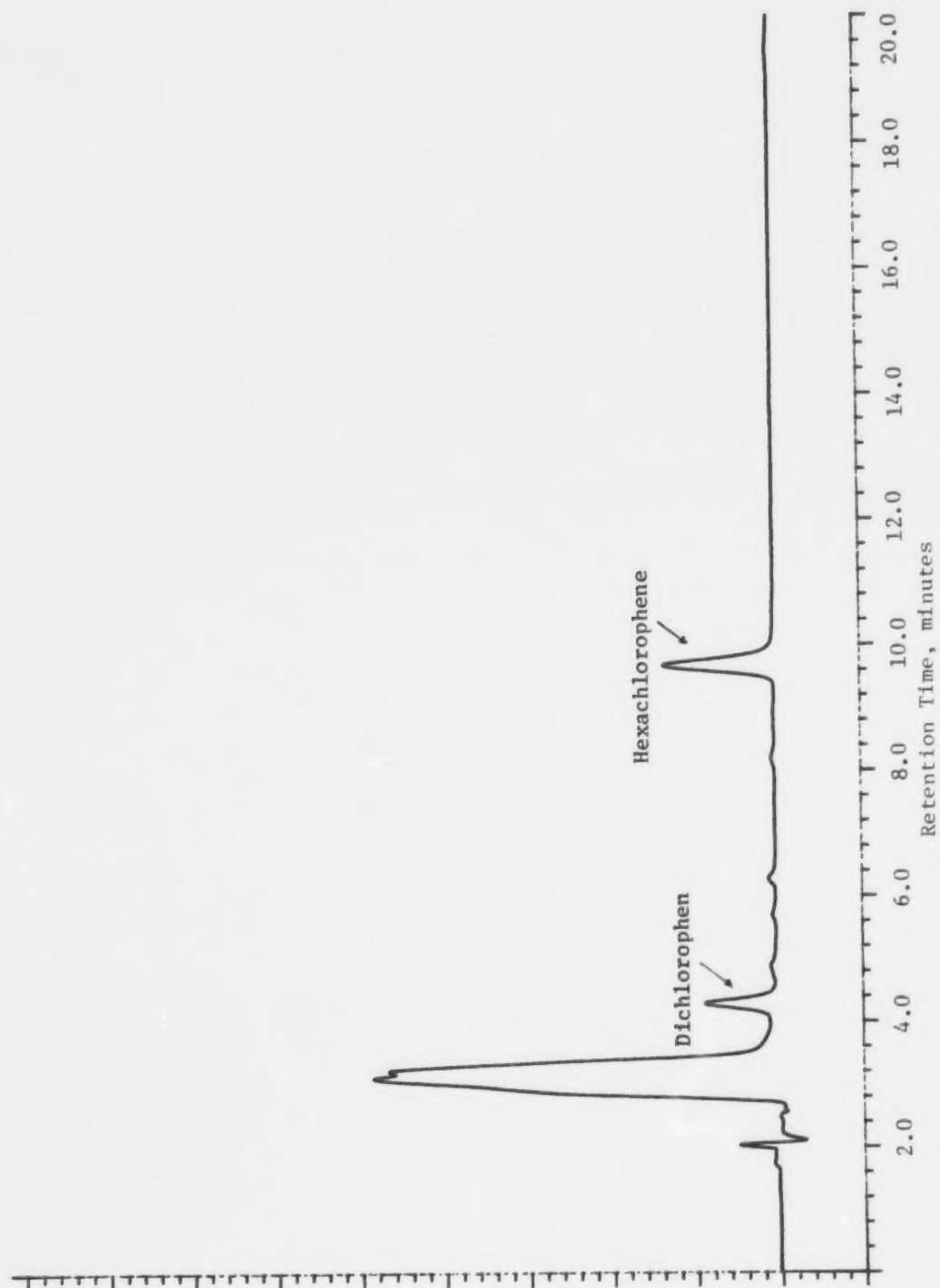


Figure 1. HPLC-UV Chromatogram of 10 ng Each of Hexachlorophene and Dichlorophen (Column 1)

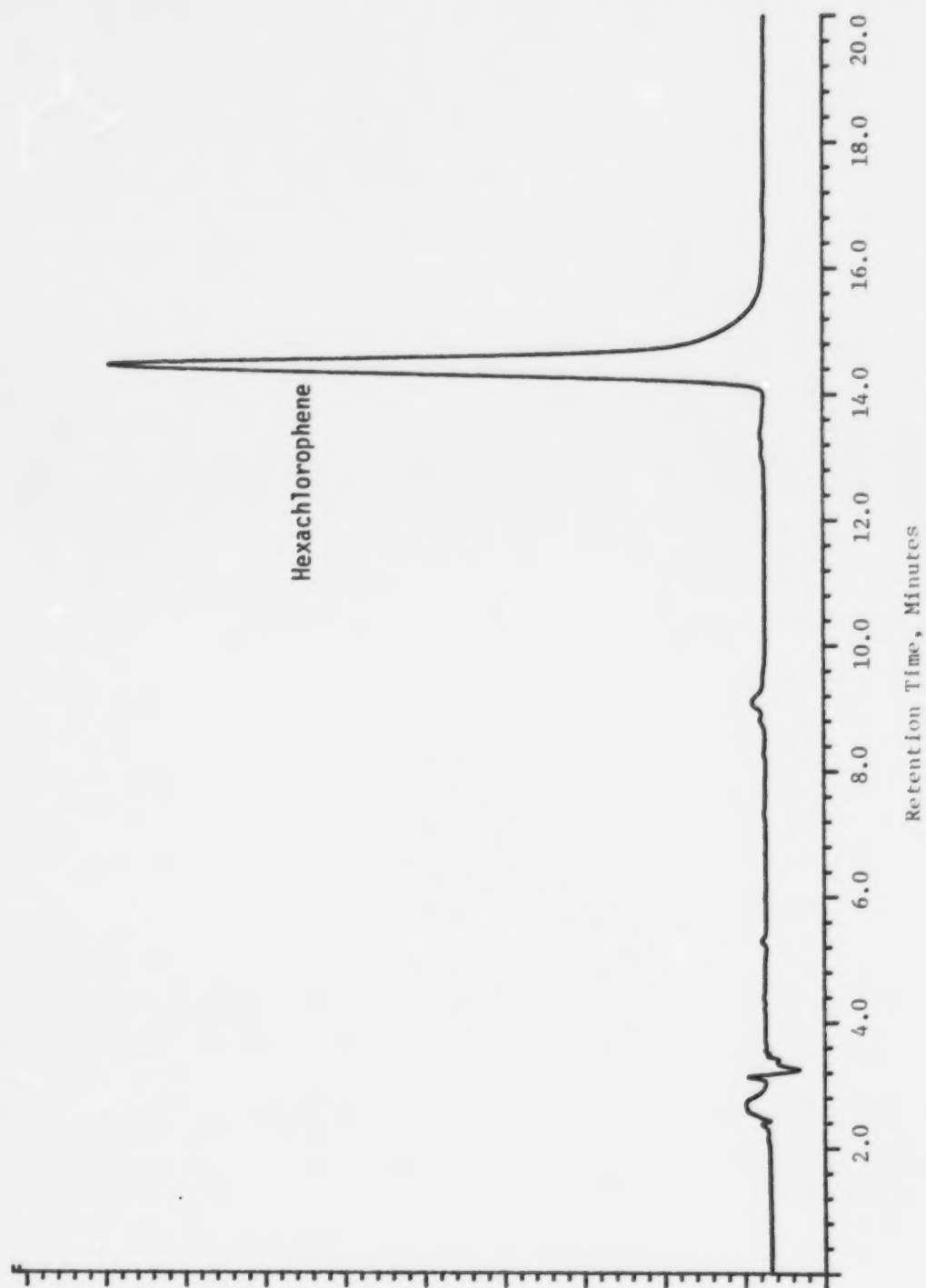


Figure 2. HPLC-UV Chromatogram of 250 ng of Hexachlorophene (Column 2)

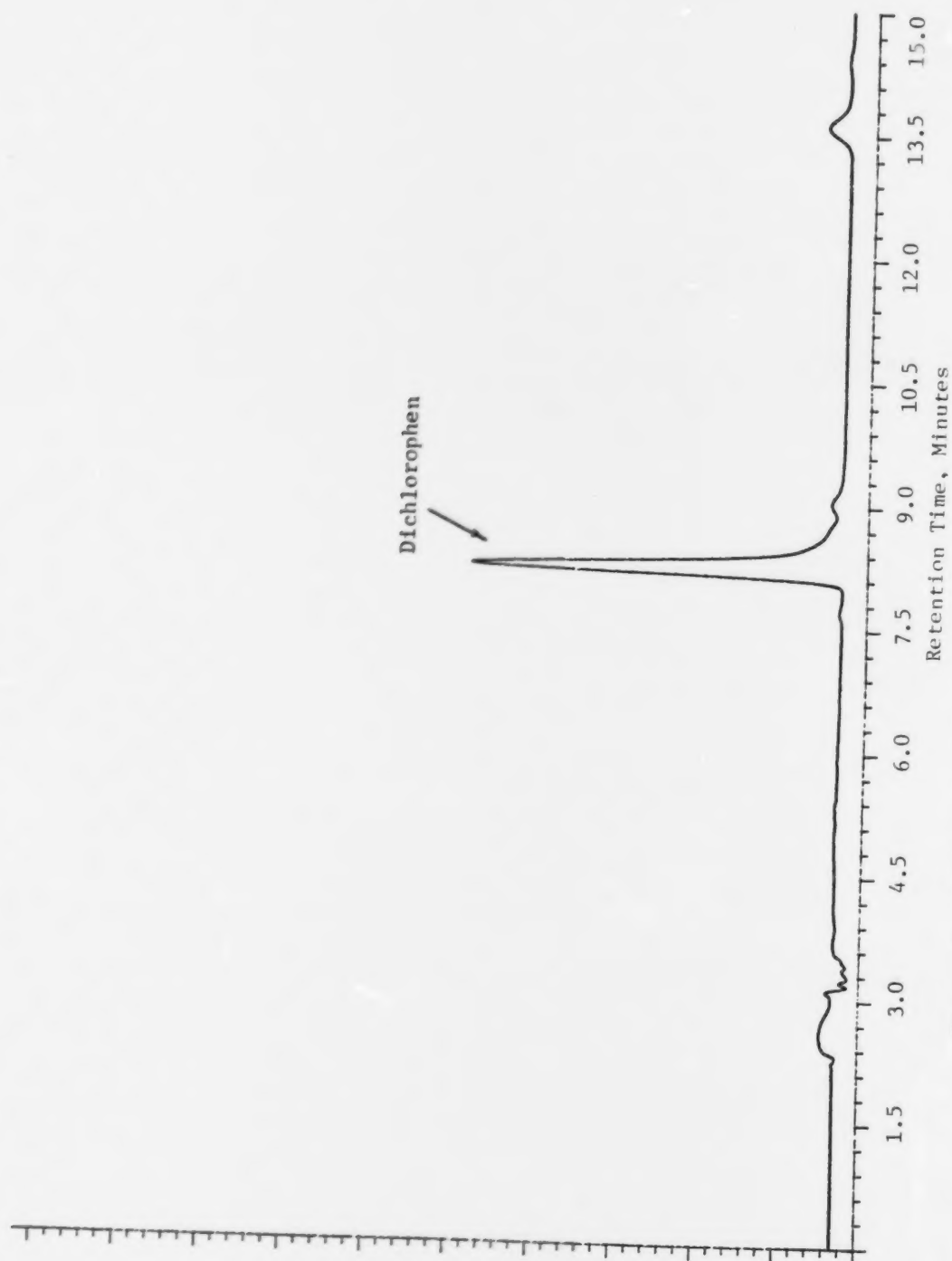


Figure 3. HPLC-UV Chromatogram of 250 ng of Dichlorophen (Column 2)

## VALIDATIONS STUDIES

Recovery of hexachlorophene from reagent water at the 4.0  $\mu\text{g/L}$  level was  $3.7 \pm 0.4$   $\mu\text{g/L}$ . The MDL in reagent water was calculated to be 1.2  $\mu\text{g/L}$ . Recoveries of hexachlorophene from reagent water at the 4.0, 10, 50, 200, and 1000  $\mu\text{g/L}$  levels were 3.7, 9.6, 50, 190, and 910  $\mu\text{g/L}$ , respectively. These data were the averages of duplicate analyses. Individual data points are given in Table 1. The resultant analytical curve is shown in Figure 4.

TABLE 1. ANALYTICAL CURVE DATA FOR HEXACHLOROPHENE AND DICHLOROPHEN IN REAGENT WATER.

| Compound        | Amount Recovered From Reagent Water at Given Spike Level, $\mu\text{g/L(a)}$ |          |        |          |          |
|-----------------|------------------------------------------------------------------------------|----------|--------|----------|----------|
|                 | 4.0                                                                          | 10       | 50     | 200      | 1000     |
| Hexachlorophene | 3.5 3.9                                                                      | 9.3, 9.9 | 48, 52 | 180, 200 | 880, 930 |
| Dichlorophen    | 3.5, 4.1                                                                     | 9.5, 9.7 | 54, 48 | 180, 220 | 890, 950 |

(a) Results of duplicate analyses.

Recoveries of hexachlorophene from Columbus POTW effluent at the 10 and 50  $\mu\text{g/L}$  levels were  $82 \pm 3$  percent and  $102 \pm 6$  percent, respectively. These data were the averages of seven replicate analyses.

Recovery of dichlorophen from reagent water at the 4.0  $\mu\text{g/L}$  level was  $3.8 \pm 0.3$   $\mu\text{g/L}$ . The MDL in reagent water was calculated to be 1.0  $\mu\text{g/L}$ . Recoveries of dichlorophen from reagent water at the 4, 10, 50, 200, and 1000  $\mu\text{g/L}$  levels were 3.8, 9.6, 51, 200, 920  $\mu\text{g/L}$ , respectively. These data were the averages of duplicate analysis. Individual data points are given in Table 1. The resultant analytical curve is shown in Figure 5.

Recoveries of dichlorophen from Columbus POTW effluent at the 10 and 50  $\mu\text{g/L}$  levels were  $58 \pm 12$  and  $107 \pm 4$  percent, respectively. These data were the averages of seven replicate analyses. The source of the low recoveries obtained from Columbus POTW effluent is not known. It may be due to some characteristic of the wastewater and may also be somewhat concentration dependent.

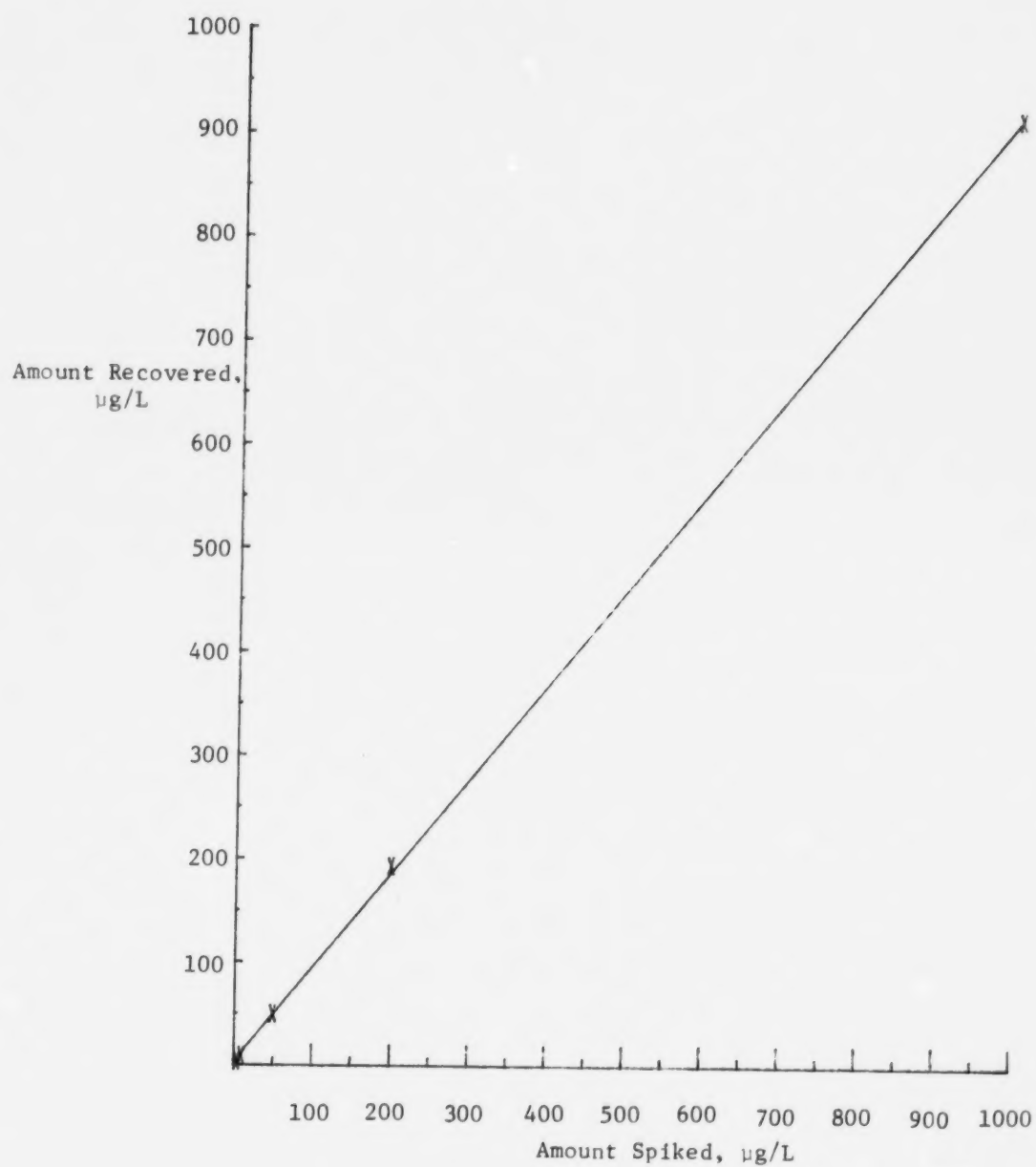


Figure 4. Analytical Curve for Hexachlorophene

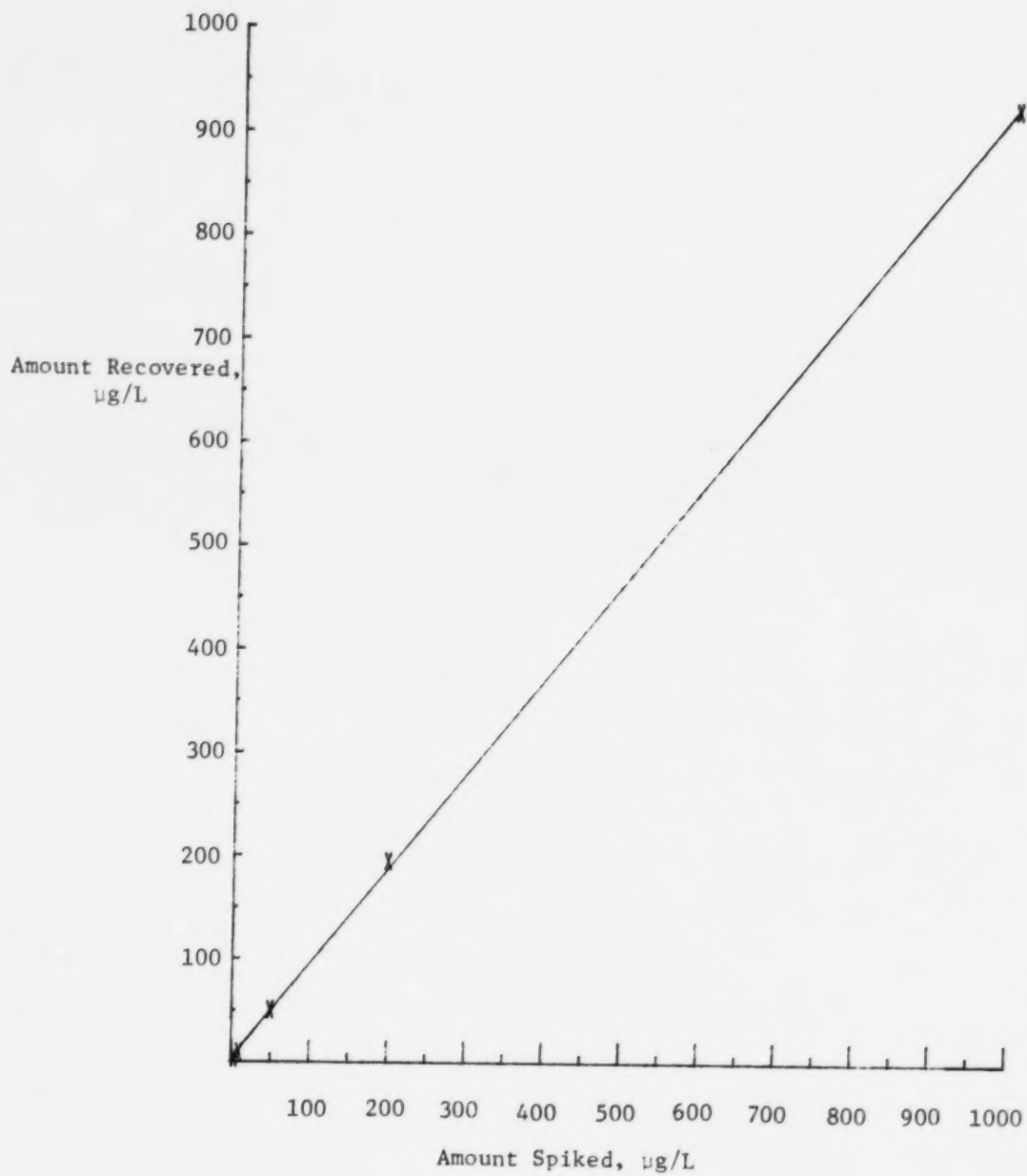


Figure 5. Analytical Curve for Dichlorophen

#### REFERENCES

1. Liem, D.H. Analysis of Antimicrobial Compounds in Cosmetics. Cosmet. Toiletries, 92(3):59-62, 67-68, 70-72, 1977.
2. Palermo, P.J. and Lundbery J.B. Simultaneous Programmed Temperature Gas Chromatographic Assay of Zinc Undecylenate, Salicylic Acid and Dichlorophen in an Aerosol Foot Powder. Can. J. Pharm. Sci., 14(1):21-3, 1979.

DETERMINATION OF HEXACHLOROPHENE AND DICHLOROPHEN IN  
INDUSTRIAL AND MUNICIPAL WASTEWATERS  
METHOD 604.1

1. Scope and Application

- 1.1 This method covers the determination of certain phenolic pesticides. The following parameters can be determined by this method:

| <u>Parameters</u> | <u>CAS No.</u> |
|-------------------|----------------|
| Dichlorophen      | 97-23-4        |
| Hexachlorophene   | 70-30-4        |

- 1.2 This is a high performance liquid chromatographic (HPLC) method applicable to the determination of the compounds listed above in industrial and municipal discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 14) for each compound is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of liquid chromatography and in the interpretation of liquid chromatograms. Each analyst must

demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

- 1.5 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second liquid chromatographic column that can be used to confirm measurements made with the primary column.

## 2. Summary of Method

- 2.1 A measured volume of sample, approximately 1 liter, is adjusted to pH 4-4.5 and solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to methanol during concentration to a volume of 5 mL or less. Liquid chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by HPLC using an ultraviolet detector (UVD).<sup>1</sup>

## 3. Interferences

- 3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in liquid chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

- 3.1.1 Glassware must be scrupulously cleaned.<sup>2</sup> Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Follow by washing with hot water

and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Some thermally stable materials such as PCBs may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The acid/base extraction cleanup described in Section 10 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

#### 4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory

- 5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)
- 5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.
  - 5.2.2 Drying column - Chromatographic column 400 mm long x 10 mm ID with coarse frit.
  - 5.2.3 Chromatographic column - 400 mm long x 19 mm ID with 250 mL reservoir at the top and Teflon stopcock (Kontes K-420290 or equivalent).
  - 5.2.4 Concentrator tube, Kuderna-Danish - 25-mL, graduated (Kontes K-570050-0250 or equivalent). Calibration must be checked at the volumes employed in the test. A ground glass stopper is used to prevent evaporation of extracts.
  - 5.2.5 Evaporative flask, Kuderna-Danish - 1000-mL (Kontes K-570001-1000 or equivalent). Attach to concentrator tube with springs.
  - 5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).
  - 5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).
  - 5.2.8 Vials - Amber glass, 5 to 10 mL capacity with Teflon lined screw-cap.
- 5.3 Boiling chips - approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or Soxhlet extract with methylene chloride.
- 5.4 Water bath - Heated, capable of temperature control ( $\pm 2^\circ\text{C}$ ). The bath should be used in a hood.
- 5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.

- 5.6 Liquid chromatograph - Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, detector and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.6.1 Pump - Isocratic pumping system, constant flow.
- 5.6.2 Column 1 - Reversed-phase column, 5 micron Spherisorb-ODS, 250 x 4.6 mm or equivalent. This column was used to develop the method performance statements in Section 14. Alternate columns may be used in accordance with provisions described in Section 12.1
- 5.6.3 Column 2 - Reversed-phase column, 5 micron Lichrosorb RP-2, 250 x 4.6 mm or equivalent.
- 5.6.4 Detector - Ultraviolet absorbance detector, 254 nm. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 14. Alternative detectors may be used in accordance with the provisions described in Section 12.1.

## 6. Reagents

- 6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.
- 6.2 Methylene chloride, methanol, acetonitrile, distilled-in-glass quality or equivalent.
- 6.3 Sodium sulfate - (ACS) granular, anhydrous. Heat in a muffle furnace at 400°C overnight.
- 6.4 Sodium phosphate, monobasic - (ACS) crystal.
- 6.5 1N Sodium hydroxide. Dissolve 4.0 grams of NaOH (ACS) in 100-mL of distilled water.

6.6 Phosphoric acid, (85%).

6.7 Stock standard solutions (1.00  $\mu\text{g}/\mu\text{L}$ )- Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.7.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

## 7. Calibration

7.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1. The liquid chromatographic system can be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

- 7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with 50/50 methanol/water. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
- 7.2.2 Using injections of 20 to 50  $\mu$ L of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.
- 7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than +10%, the test must be repeated using a fresh calibration

standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with 50/50 methanol/water. One of the standards should be a representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 20 to 50  $\mu$ L of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_s C_{is}) / (A_{is} C_s)$$

where:

$A_s$  = Response for the compound to be measured.

$A_{IS}$  = Response for the internal standard.

$C_{IS}$  = Concentration of the internal standard in  $\mu\text{g/L}$ .

$C_S$  = Concentration of the compound to be measured in  $\mu\text{g/L}$ .

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios,  $A_S/A_{IS}$  against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than  $\pm 10\%$ , the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

## 8. Quality Control

8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.

- 8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
  - 8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations.
  - 8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

- 8.2.3 Calculate the average percent recovery ( $R$ ), and the standard deviation of the percent recovery ( $s$ ), for the results. Wastewater background corrections must be made before  $R$  and  $s$  calculations are performed.
- 8.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1 Calculate upper and lower control limits for method performance as follows:
- $$\text{Upper Control Limit (UCL)} = R + 3s$$
- $$\text{Lower Control Limit (LCL)} = R - 3s$$
- where  $R$  and  $s$  are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts<sup>6</sup> that are useful in observing trends in performance.
- 8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as  $R \pm s$ . The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of  $R$  and  $s$ . Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in

Section 8.4. The accuracy statements should be updated regularly.<sup>6</sup>

- 8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5 Before processing any samples, the analyst should demonstrate through the analysis of a 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.
- 8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as liquid chromatography with a dissimilar column, must be used. Whenever possible, the laboratory

should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Samples Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices<sup>7</sup> should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 Adjust the pH of the sample to 6 to 8 with sodium hydroxide or sulfuric acid immediately after sampling.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel.

10.2 The analyst may solvent wash the sample at basic pH as described in Sections 10.2.1 and 10.2.2 to remove potential method interferences. For relatively clean samples the wash should be omitted and the extraction, beginning with Section 10.3, should be followed.

10.2.1 Adjust the pH of the sample to 12.0 with 1N sodium hydroxide.

10.2.2 Add 60 mL of methylene chloride to the separatory funnel and extract the sample by shaking the funnel for 2 min

with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 min. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Discard the methylene chloride extract. Perform a second and third extraction in the same manner.

- 10.3 Add 50 g of  $\text{NaH}_2\text{PO}_4$  to the sample in the separatory funnel and shake to dissolve the solid. The sample pH should be between 4.0 and 4.5. If necessary, adjust the pH with phosphoric acid or sodium hydroxide. Add 200 mL of methylene chloride to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separations. Collect the methylene chloride extract in a 1 L Erlenmeyer flask.
- 10.4 Add a second 200 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.5 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 25-mL concentrator tube to a 1000-mL evaporative flask. Other

concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.6 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 ml of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 ml of methylene chloride.

10.7 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in approximately 60 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reached 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

10.8 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 15 ml of methanol and a new boiling chip and attach a micro-Snyder. Pour about 1 mL of methanol into the top of the micro-Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 min. When the apparent volume of liquid reaches

1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 min.

10.9 Remove the micro-Snyder column and adjust the volume to 2.5 mL with methanol. Transfer the liquid to a 5-mL volumetric flask and dilute to the mark with reagent water. Mix thoroughly prior to analysis. If the extracts will not be analyzed immediately, they should be transferred to Teflon sealed screw-cap vials and refrigerated. If the sample extract requires no further cleanup, proceed with liquid chromatographic analysis. If the sample requires additional cleanup, proceed to Section 11.

10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

## 11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method, namely the acid/base extraction described in Section 10, has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of additional cleanup, the analyst must demonstrate that the recovery of each compound of interest is no less than 85%.

## 12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separation achieved by Column 1 is shown in Figure 1. Examples of the separation achieved by Column 2 are

shown in Figures 2 and 3. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met

12.2 Calibrate the liquid chromatographic system daily as described in Section 7.

12.3 If an internal standard approach is being used, the analyst must not add the internal standard until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 20 to 50  $\mu$ L of the sample extract by completely filling the sample valve loop. Record the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

### 13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak

response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V<sub>i</sub> = Volume of extract injected in  $\mu\text{L}$ .

V<sub>t</sub> = Volume of total extract in  $\mu\text{L}$ .

V<sub>s</sub> = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(\text{RF})(V_0)}$$

where:

A<sub>s</sub> = Response for the compound to be measured.

A<sub>is</sub> = Response for the internal standard.

I<sub>s</sub> = Amount of internal standard added to each extract in  $\mu\text{g}$ .

V<sub>0</sub> = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

#### 14. Method Performance

- 14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.<sup>8</sup> The MDL concentrations listed in Table 1 were obtained using reagent water.<sup>1</sup> Similar results were achieved using representative wastewaters.
- 14.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 10 x MDL to 1000 x MDL.
- 14.3 In a single laboratory, Battelle's Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 2.<sup>1</sup>

## REFERENCES

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TABLE 1. CHROMATOGRAPHIC CONDITIONS AND METHOD DETECTION LIMITS

| Parameter       | Retention Time (Min) |          | MDL<br>( $\mu\text{g/L}$ ) |
|-----------------|----------------------|----------|----------------------------|
|                 | Column 1             | Column 2 |                            |
| Dichlorophen    | 4.2                  | 8.2      | 1.0                        |
| Hexachlorophene | 9.7                  | 14.4     | 1.2                        |

Dichlorophen, Hexachlorophene

Column 1 conditions: Spherisorb-ODS, 5 micron, 250 x 4.6 mm; 1 mL/min flow; 65/35 acetonitrile/water, 0.05%  $\text{H}_3\text{PO}_4$ . A UV detector was used with this column to determine the MDL.

Column 2 conditions: Lichrosorb RP-2, 5 micron, 250 x 4.6 mm; 1 mL/min flow; 50/50 acetonitrile/water, 0.5 acetic acid.

TABLE 2. SINGLE LABORATORY ACCURACY AND PRECISION(a)

| Parameter       | Sample Type(b) | Background, $\mu\text{g/L}$ | Spike Level $\mu\text{g/L}$ | Mean Recovery (%) | Standard Deviation (%) | Number of Replicates |
|-----------------|----------------|-----------------------------|-----------------------------|-------------------|------------------------|----------------------|
| Dichlorophen    | 1              | ND(c)                       | 10                          | 58                | 12.4                   | 7                    |
|                 | 1              | ND                          | 50                          | 107               | 3.9                    | 7                    |
| Hexachlorophene | 1              | ND                          | 10                          | 82                | 2.7                    | 7                    |
|                 | 1              | ND                          | 50                          | 102               | 5.8                    | 7                    |

(a) Column 1 conditions were used.

(b) 1 = POTW secondary effluent.

(c) ND = Not detected.

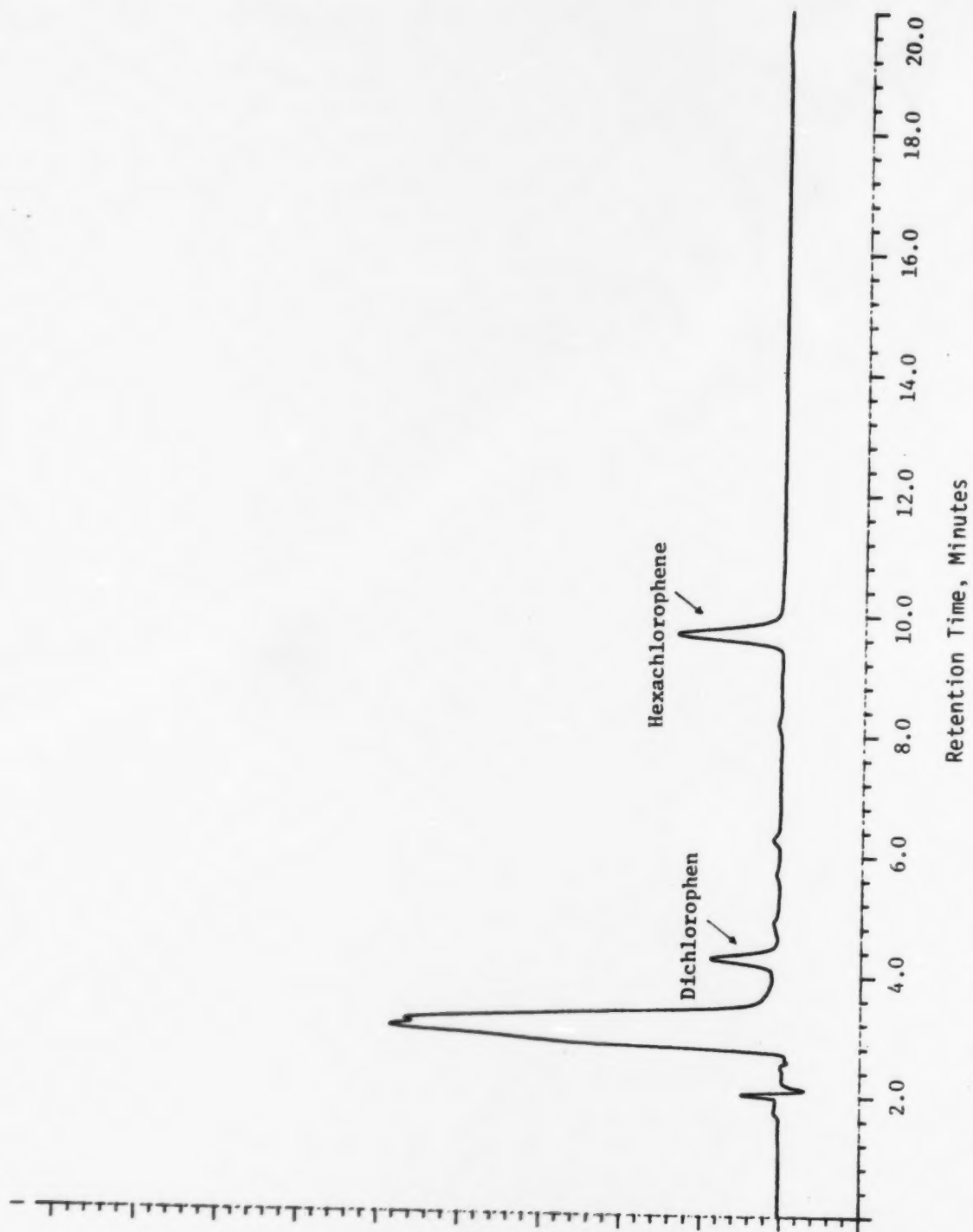


Figure 1. HPLC-UV Chromatogram of 10 ng Each of Hexachlorophen and Dichlorophen (Column 1).

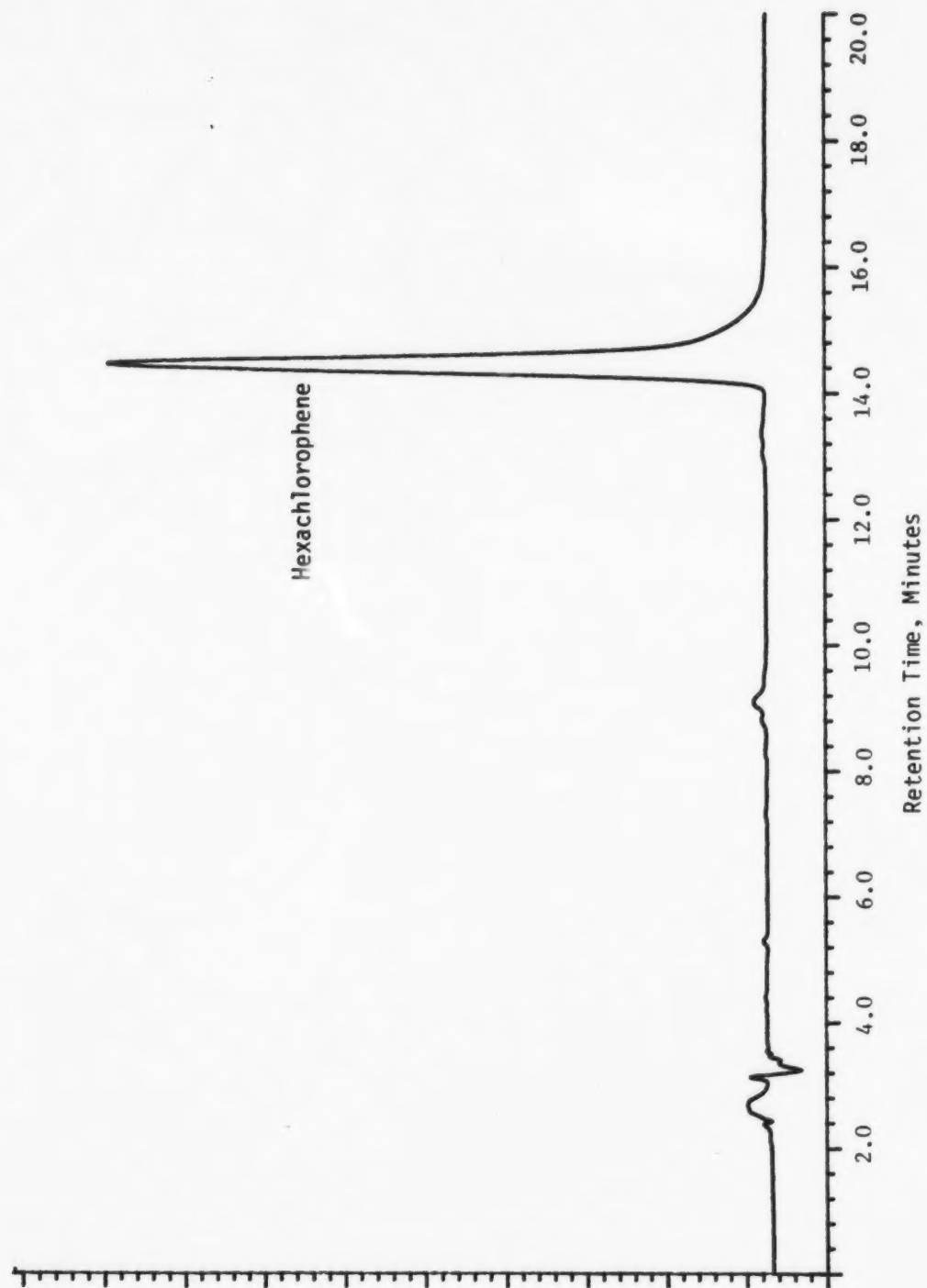


Figure 2. HPLC-UV Chromatogram of 250 ng Each of Hexachlorophene (Column 2).

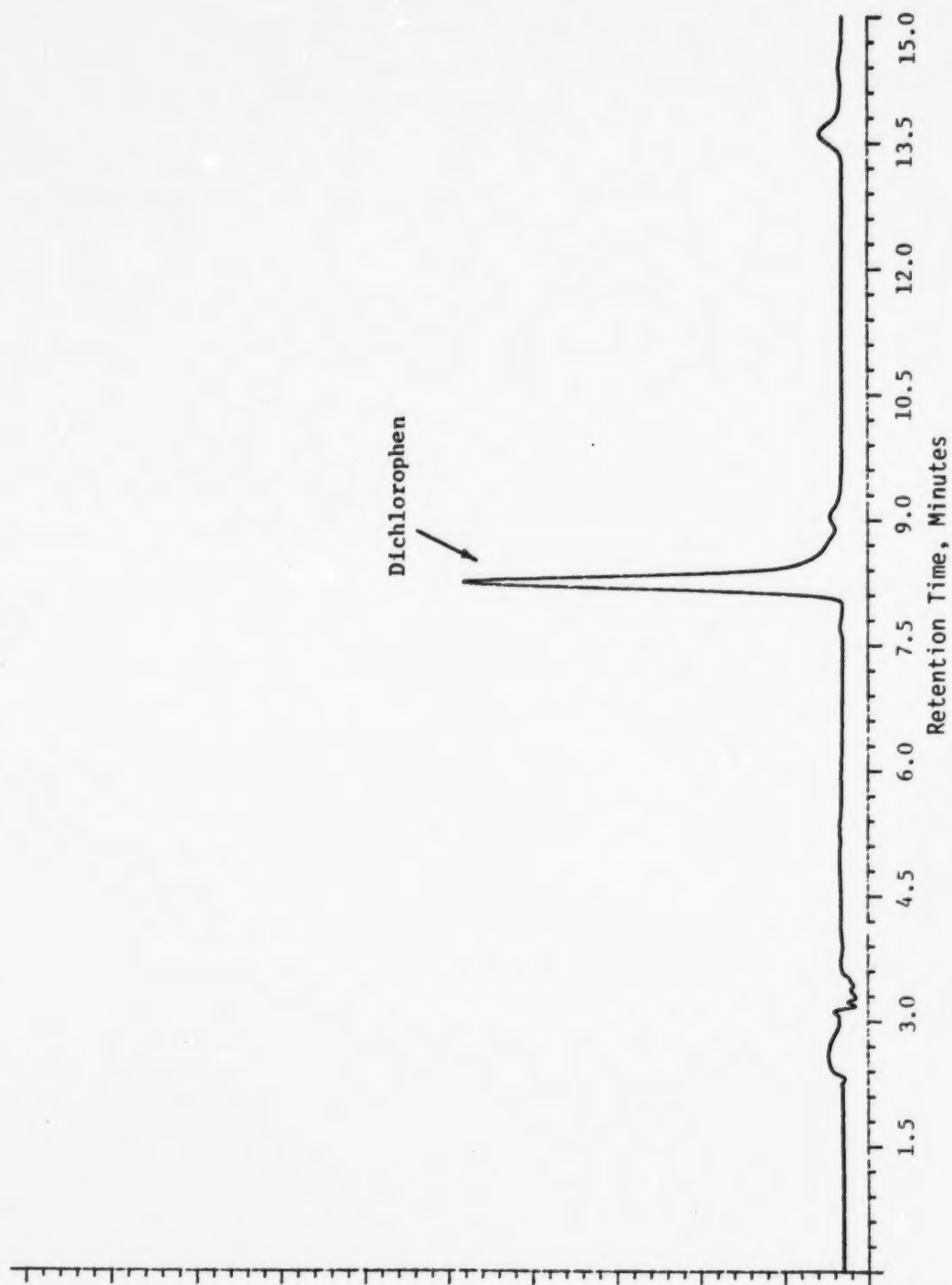


Figure 3. HPLC-UV Chromatogram of 250 ng of Dichlorophene (Column 2)